THE EFFECTS OF FEEDING *NIGELLA SATIVA L.*ON SOME IMMUNE REACTIONS AND THE LEVELS OF BLOOD PROTEINS IN DIABETIC RABBITS

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Dedications

To my parents for their support and encouragement to see me successful in life.

To my brothers, sisters and friends for their continuous moral support and care.

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CONTENTS

List of Tables

List of Figures

ABSTRACT

This study to elucidate the effect of *N. sativa* on some biochemical parameters such as glucose, proteins, albumin and globulins, and to investigate the effect of *N. sativa* seeds on some immune functions by studying the phagocytic activity and hypersensitivity.

Sixteen local male rabbits of one and half years of age , were divided into four groups A, B, C, D .Groups A and B ,non-diabetic , C and D are diabetic. All groups were fed on sorghum. Groups B and D replaced their diet with 15 % of black cumin seeds. The treatment continued for one month from August to September (2004). Serum samples were collected every two weeks for determination of glucose, total proteins, albumin and globulins. Phagocytic activity and hypersensitivity tests were done at the end of the experiment.

The statistical analysis of data revealed that feeding 15 % of black cumin to non-diabetic and diabetic rabbits leads to non significant reduction in glucose level, Significant increased in total serum proteins and albumin ($P < 0.05$),and non significant increasing in globulins. The increasing effect appeared after treatment for two weeks and continued until week 4 in total proteins and albumin.

Results showed significant rise in polymorph nuclear leukocyte ($P < 0.05$), and anti- inflammatory effect of *N. sativa* observed by non significant decrease in skin thickness in treated groups.

خلاصة الاطروحة

أجريت التجربة لتوضيح أثر الحبة السوداء على بعض المؤشرات الحيوية مثل جلكوز الدم ، البروتينات الكلية ، والقلوبينات الكلية. ولدراسة أثر الحبة السوداء على بعض وظائف الجهاز المناعي مثل البلعمة وإختبار الحساسية المفرطة.

تم إختيار 16 أرنب (ذكور) يتراوح عمرها ما بين السنة و السنة ونصف ، تم تقسيمها الى أربعة مجموعات أ ، ب ، ج ، د.

المجموعتين (أ) و (ب) غير مصابتين بمرض السكري والمجموعتين (ج) و (د) مصابتين بمرض السكري . تم تغذية المجموعتين (ب) و (د) على الحبة السوداء وذلك باستبدال 15 % من وجبة الذرة ببذور الكمون الأسود ، أما المجموعتين (أ) و (ج) تمت تغذيتهم على الذرة فقط.

استمرت معالجة الأرانب بالحبة السوداء لمدة شهر من أغسطس الى سبتمبر.

تم جمع عينات مصل الدم كل اسبوعين لتحديد المؤشرات الحيوية ، وفي نهاية التجربة جمعت عينات الدم لتحديد النشاط البلعمي وتم قياس سمك الجلد لإختبار الحساسية المفرطة.

كشف التحليل الإحصائي للنتائج بأن تغذية الأرانب – المصابة وغير المصابة بمرض السكري - بالحبة السوداء ادى الى نقصان غير معنوي في جلكوز الدم ، وزيادة معنوية في مستوى تركيز كل من البروتينات الكلية والاليبومين وزيادة غير معنوية في مستوى تركيز القلوبينات الكلية.

ظهر أثر الحبة السوداء على مستوى جلكوز الدم بعد إسبوعين واستمر حتى الإسبوع الرابع في المجموعة المعالجة بالكمون الأسود وغير مصابة بالسكري (المجموعة ب).

ظهر أثر الحبة السوداء على البروتينات الكلية والاليبومين والقلوبينات الكلية بعد معالجتها لمدة اسبوعين وإستمرت الزيادة الى الأسبوع الرابع.

أوضحت النتائج زيادة معنوية في كريات الدم البيضاء ونقصان غير معنوي في سمك الجلد في إختبار الحساسية المفرطة عند المجموعات المعالجة.

Introduction

Nigella sativa L. (black cumin), which belongs to the Ranuculaceae family, is an erect annual herb. It is cultivated in different parts of the world and is especially grown in East Mediterranean countries. Other varieties present are *N. damascrea* (Turkish black cumin) and *Nigella arvensis* (field black cumin / wild black cumin).
In Sudan it is mainly cultivated in Northern Sudan and Darfur (El-Moniem *et al*., 2005).

N. sativa is considered as one of the famous medicinal plants, used for curing many diseases since ancient times. This may be related to the famous say of Prophet Mohammed about black cumin that it treats every illness except death.

The seeds oil has anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity (Ali and Blunden, 2003).Also they are used for treating various allergies (Kalus *et al*., 2003). *N. sativa* used as natural medicine for treatment of many acute, as well as, chronic conditions; these include diabetes, hypertension and dermatological conditions (Kirui *et al*., 2004). Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration – hyperglycemia – due to insulin deficiency and / or insulin resistance, insulin is the main hormone controlling intermediary metabolism, having action on carbohydrates, fat and protein metabolism.

Antidiabetic properties of *N. sativa* seeds were suggested to be mediated by stimulating of insulin release (Rchid *et. al*., 2004) .Many authors proved that the *N. sativa* seeds reduce blood glucose concentration (El-Dakhankhany *et al*.,2002) and increase total serum proteins and its two fractions albumin and total globulins level (Al-Gaby,1998) .

Scientific data on using the whole *N. sativa* seeds are scarce. Previous studies on *N. sativa* concentrated in using *N. sativa* oil extract or its use as mixture with other medicinal plants. Their studies concerned with effects of *N .sativa* on hyperglycemia caused by diabetes mellitus, without investigating its effect on the immune response in

diabetic patients as it is well known that acute, short –term hyperglycemia affects all major components of innate immunity and impairs the ability of the host to combat infection (Turina *et al* ., 2005) .

This work is designed to study the effect of feeding diabetic rabbits with *N. sativa* seeds alone without being mixed with other medicinal plants, compared with non diabetic ones, on some immune reactions and on the plasma protein levels specially the concentration of the total globulins fractions.

CHAPTER ONE

LITERATURE REVIEW

1.1 *Nigella sativa*

The seeds of *Nigella sativa*, commonly known as black cumin, are used in herbal medicine all over the world, in the treatment and prevention of number of diseases and conditions that include asthma, diarrhea, and dyslipidaemia (Ali and Blunden, 2003).

1.1.1 Chemical Composition of *Nigella sativa*

The chemical composition of the extracted fixed oil (total fatty acid composition) and volatile oil were determined by Gas Chromatography. Eight fatty acids (99.5%) and thirty – two compounds (86.7%) had been identified in the fixed and volatile oils, respectively. The main fatty acids of the fixed oils; were Linoleic acid (55.6%), oleic acid (23.4%) and Palmitic acid (12.5%). The major compounds of the volatile oils were p-cymene (14.08%), Limohne (4.3%) and Carvone (4.0%) (Nickavar, *et al*.,

1.1.2 Fixed and Volatile Oils

Twenty compounds were identified in the oil of black cumin seed ,obtained in 0.4% (v/w) yield, it contains both para-cymene (37.3%) and thymoquinone (13.7%) which were the major components .(Hajhashemi *et al*., 2004).

1.1.3 Some Biological Activities of *N. sativa*

The pharmacological actions of the crude extraction of *N. sativa* seeds (and some of its active constituents e .g: volatile oil and thymoquinone) that have been reported have protection against nephrotoxicity and hepatotoxicity induced by either disease or chemical .Anti-inflammatory, analgesic, antipyretic, antimicrobial and anti-neoplastie activity also was investigated (Ali and Blunden, 2003).

1.1.3 .1 Antibacterial and Antifungal properties

N. sativa showed antimicrobial activity especially for the gram negative bacteria (Morsi, 2000).Another study by El-Fatatry (1975) showed the antimicrobial activity of the volatile oil against gram positive microorganisms. The essential oils of *N. sativa* seeds, *C. citratus* leaves and *P. undulata* aerial parts (Collected from Sudan) were screened to anti –bacterial properties. All essential oil exhibited activity against *Staphylococcus aureus*, Bacillus, *E. coli* and pseudomonas (Elkamali *et al.,* 1998).

Anti-fungal activity against seven pathogenic fungal strains : *Aspergillus niger ,A. flavus , A .fumigatus ,A .tyerreus ,Candida albicans* ,Madurellagrisea , and *M.mycetommatis*, the volatile oils exhibited complete inhibition of most fungal strains used (El- Moniem, *et al*.,2005). Similar results were founded by (Khan *et al*., 2003).

1.1.3.2 Enhancement of bone healing

In study carried by (kirui *et a*l ., 2004) on active components of black seed, Thymoquinone (TMQ) at sustained level, revealed that sustained levels of (TMQ) can enhance bone healing .

1.1.3.3 Anti-cancer effect

Thymoquinone (TQ) the most abundant constituent present in black seed, inhibits the growth of colon cancer cells which was correlated with G1phase arrest of the cell cycle. This supports the potential using of TQ for treatment of colon cancer. (Gali *et al.,* 2004).

1.1.3.4 Anti – oxidant effect

Treatment with *N. sativa* increased the antioxidant defense system activity by increasing the level of glutathione (GSH), also prevented lipid peroxidation (Meral *et a*l.,2001) . *N. sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5- lipoxygenase products in polymorphonuclear leukocytes (El-Dakhankhany *et al*.,2002)

1.1.3.5 Effect of *N. sativa* on hemoglobin level

Treatment of rats with the seed extract for up to 12 weeks has been reported to induce changes in the heamogram such as induce increased in the packed cell volume (PVC) and hemoglobin (Hb) and decreased cholesterol , triglycerides and glucose levels (Ali and Blunden, 2003).

1.1.3.6 Oxidative stress

N. sativa treatment exerts therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta cell integrity. Consequently, *N. sativa* may be clinically useful for protecting beta cell against oxidative stress (Kanter *et al.,* 2004) .

1.1.3.7 Anorexic effect and insulin – sensitizing actions

Petroleum ether extract of *N. sativa* has a slight anorexic effect, and that it contains the hypolipidemic activity previously obtained with the plant. In vivo treatment with the petroleum ether extract exert an insulin – sensitizing actions by enhancing the activity of the two major intracellular signal transduction pathways of the hormone's receptor (Le *et al.,*2004)

1.1.3.8 Stimulation of insulin release

Richid *et al.,* (2004) Reported that different fractions of *N. sativa* seeds were prepared, the first containing acidic and neutral compounds, the second containing basic compounds, the result showed that addition of whole extract of the basic sub-fraction of the seed to isolated rat pancreatic islets in the presence of

8.3 m mol\ glucose per liter, in this incubation medium significantly increased glucose – induced insulin release from the Islet.

1.2 Diabetes Mellitus

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficient of insulin. This can arise in many different ways, lack of insulin affects the metabolism of carbohydrates, proteins and fat, and causes significant disturbance of water and electrolytes homeostasis. Death may result from acute metabolic decomposition, while long – standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, with those at the vascular system being particularly susceptible. These changes lead to the development of well defined clinical entities, so-called complication of diabetes which characteristically affects the eyes, kidneys, and the nervous system (Ganong, 1983).

In diabetes mellitus the function of neutrophil suppressed ,therefore produces less free oxygen radicals and reduces phagocytosis and intracellular killing of candida cells associated with this reduced oxygen generation during infection (Ueta*, et al*., 1993).

1.2.1 Classification of Diabetes

There are two major clinical classes of diabetes mellitus: type I or insulin – dependent diabetes mellitus (IDDM) and type 2 or non-insulin dependent diabetes mellitus (NIDDM). Type2 diabetes in which resistance to the effect of insulin or defect in insulin secretion may be seen (Robert *el al.,* 1999).

1.2.2 Diabetogenic agents

Diabetes can be produced by the administration of certain anteriorpituitary extracts or hormones and also by the intravenous injection of alloxan, which leads to selective necrosis of the B- cells of the pancreatic islet, the insulin secreting cells. With those methods producing diabetes, the secretion of pancreatic juice and intestinal digestion remain undistributed. Experimentally produced diabetes regardless of the means

employed, is not of the same severity in all species of animals. On the rabbit it exhibits mild forms of diabetes (Dukes, 1955).

1.2.3 Alloxan action

Alloxan is widely used to induce experimental diabetes in animals. The mechanism of its action in B- cell of pancreas was investigated by (Sz kudelski, 2000). The cytotoxic action of this daibetogenic agent was mediated by reactive oxygen species. However, the source of generation is alloxan and the product of reduction, dialuric acid, establish aredox cycle with the formation of superoxide radicals. Consequently hydrogen peroxide and hydroxyl radicals are generated. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B – cells.

1.2.4 Insulin Action

Diabetes mellitus is due to insufficient action of insulin owing either to its absence or to resistance to its action. The central role of insulin is in carbohydrate, lipid, and protein metabolism. So insulin deficiency leads to decreased glucose uptake, increased glucose production by the liver gluconeogenesis, increased protein catabolism and in increase lipolysis (Robert *el al.,* 1999).

1.3 Blood Parameters

1.3.1 Glucose

Glucose is a major fuel of the tissues of mammals and universal fuel of the fetus. Glucose utilization is influenced by insulin. In normal person, the glucose ingested is converted to energy by the glycolytic pathway and about half of the ingested glucose is stored as glycogen or fat. In the absence of insulin (hyperglycemia) the anabolic processes of glycogensis and lipogenesis are impeded (Robert *et al*., 1999). Hypoglycemic effect of *N. sativa* was elucidated by (Fararh *et al.,* 2004) and its effect

mediated through decreased hepatic gluconeogenesis. The same result was obtained by (Al-awadi *et al.,* 1991) who they used *N. sativa* with other plant mixture extract. El-Dakhankhany *et al*. (2002) reported that the hypoglycemic effects of *N. sativa* oil may be mediated by extra pancreatic actions rather than by stimulated insulin release. Other finding by (Fararh *et al*., 2002) showed that *N. sativa* had hypoglycemic effect on diabetic hamsters; the plant has a stimulatory effect on beta cell function with a consequent increase in serum insulin level.

1.3.2 Total serum Proteins

Proteins are essential structural components of all cells. They are important for maintaining the out put of essential secretions such as digestive enzymes and peptide or protein hormones. Proteins are also needed to synthesize plasma proteins, which are essential for maintaining osmotic balance, transporting substances through the blood, and maintaining immunity. Excess protein is treated as source of energy, with the glycogenic amino acids being converted to glucose and the ketogenic amino acids converted to fatty acids and ketoacids. .The total plasma protein is about 7 – 7.5 g/dl. Thus, the plasma proteins comprise the major part of the solids of the plasma. The proteins of the plasma are actually a very complex mixture which includes not only simple proteins but also mixed or conjugated proteins such as glycol proteins and various types of lipoproteins. The serum protein includes mainly the albumin and globulin fractions (Devlin, 1991).The plasma proteins can be classified into three major groups: fibrinogen, albumin and globulins. Most plasma proteins are synthesized in the liver. Gama – globulin are synthesized in plasma cells, and certain plasma proteins are synthesized in other sites such as endothelial cells (Ganong, 1983).The levels of certain proteins in plasma increase during acute inflammatory states or secondary due to certain types of tissue damage. These proteins are called acute phase proteins (Robert *et al*., 1999).

Al-Gaby (1998) showed that the growth and the total serum proteins and their two fractions albumin and globulin of rats are significantly increased after supplementing *N. sativa* as high protein diet.

1.3.3 Serum Albumin

Albumin is the major protein of human plasma, some albumin is present in plasma and other is present in extracellular space. Albumin is synthesized by the liver. Its important function is that it can bind and transport various ligands. These include free fatty acid (FFA), calcium certain steroidal hormones, bilirubin, and some of the plasma tryptophan (Robert *et al.,* 1999). Serum albumin is the most abundant plasma protein. One molecule of serum albumin can carry up to 10 molecules of free fatty acid (FFA), releasing them at the consuming tissue where they are taken up by passive diffusion (David and Michael, 2000).

In normal adult human, the plasma albumin level is 3.5 – 5.0 g/dl. The degraded albumin is replaced by hepatic synthesis. Albumin synthesis is regulated and it decreases during fasting and increases in such condition as nephrosis in which there is excessive albumin loss (Ganong, 1985).

1.3.4 Serum Total Globulins

Total serum globulins represent the sum of immunoglobulins and non immunoglobulins. Many non- immunoglobulins are acute phase reactions whose hepatic production is increased in response to systemic inflammatory disease. The acute phase alpha – globulins and beta globulins are synthesized in the liver. (Feldman, 2001).

1.4 Immune system

The immune system comprises several cell types , which form a net work of interacting elements that together generate humoral immunity (B- lymphocytes) , cell mediated immunity (T-lymphocytes) , and non specific immunity (monocytes and poly morph nuclear neutrophil leukocytes (PMNs).

The immune system is an important defense against microbial pathogens (William, 1990).

1.4.1 Humoral Immunity

Involve the activation of two groups of proteins that play a key role in the body's resistance to infection. These proteins help to trap and kill non self cells before they invade a self cell (David and Micheal, 2000).

1.4.2 Immunoglobulins

Immunoglobulins or antibodies produced by B- lymphocytes, Which are classified into five classes including IgA , IgD , IgE , IgG , and IgM . Each class plays different role in binding to specific non self antigen.

Cytokines are immune system proteins known as biological response modifiers. There are two types of cytokines:

(1) Monokines : are produced by tissue macrophage and include Alfa –interferon, beta-interferon,interleukins (IL-1) , tumor necrosis factor (TNF) , and several of the colony stimulatory factors (CSFs) .

(2) Lymphokines: are soluble proteins that are released from activated lymphocytes, particularly from T- cell and natural killer cell. (Abbas and Lichtman,2001).

N. sativa enhanced the production of interleukin -3 and interleukin-1B by human lymphocytes, suggesting therefore, that it has an effect on macrophages (Haq *et a*l., 1995).

1.4.3 Cell mediated immunity

Mediated by T- lymphocytes and macrophages which are present through the body. When these cells encounter the antigen on cells from another individual or the antigen on tumor cells or viruses, they bare activated (Ganong, 1985).

One of the important groups of leucocytes is the phagocytic cells, such as the monocytes, macrophages and poly-morphoneutrophils. These cells bind to

microorganisms, internalize them and then kill them .Because they use primitive non specific recognition systems which allow them to bind to a variety of microbial products, phagocytic cells mediated innate immune responses and act as a first line of defense against infection (Roitt, *et al.,* 1993).

1.4.5 Poly morphonuclear neutrophil

Poly morphonuclear neutrophils often just called neutrophils or (PMNs) are another important group of phagocytes. Neutrophils constitute the majority of the blood leucocytes and develop from the same early precursors as monocytes and macrophages. They migrate into tissues, particularly at site of inflammation, but neutrophils are considered as short lived cells, which engulf material, destroy it and then die (Roitt *et al.,*1993).

1.4.6 Monocytes

Monocytes or mononuclear phagocytes, are less abundant than neutrophil, which are derived from bone marrow stem cells.

Unlike neutrophils, monocytes differentiate into cells called macrophages.

The function of blood monocytes and tissue macrophages is to engulf particles, including infection agents, internalize and destroy them. (Abbas and Lichtman,2001)

1.4.7 Macrophages

Macrophages originate in the bone marrow.Immature macrophages called monocytes, are found in the blood. Mature macrophage are found in connective tissue, brain and lungs (Tizard, 2000)

1.5 Phagocytosis

Is one type of endocytosis, occurs only in specialized cells such as macrophages and granulocytes (polymorph nuclear leukocytes, PMNs).

Phagocytosis involves the ingestion of large particles such as viruses, bacteria, cells or debris. Macrophages are extremely active in this regard (Robert *et al.,* 1999).

When bacteria invade the body, the bone marrow is stimulated to produce and release large numbers of neutrophils. Bacterial products interact with plasma factors and cells to produce agent that attract neutrophil to the infected area (chemo taxis) .The chemo tactic agent include components of the complement system like leuktrienes and polypeptides from lymphocytes, mast cells, and basophiles.

Other plasma factors act on the bacteria to make them tasty to phagocytes (opsonization), by coating the bacteria with immunoglobulin of a particular class (IgG) and complement proteins. The neutrophils then actively ingest bacteria (phagocytosis). (Ganong, 1983).

N. sativa enhanced the production of IL-3 by human lymphocytes when used with or without stimulator; it increased IL-B suggesting that, it has effect on macrophages stimulation (Haq *et al.*, 1995).

Whole *N. sativa* and its purified proteins were found stimulatory as well as suppressive. The stimulatory effect of whole *N. sativa* and fractionated proteins was also noticed on the production of TNF-α. (Haq, *et al*., 1999).

1.6 Hypersensitivity

When some antigens are injected into the skin of the sensitized animals, slowly developing inflammatory response may occur at the injection site. Since this delayed hypersensitivity reaction can only be transferred from sensitized to normal animals by lymphocytes, it must be cell mediated reaction.

Delayed hypersensitivity reaction are classified as type IV hypersensitivity and result from interactions between the injected antigen, antigen presenting cells, and T-cells. An important example of delayed hypersensitivity reaction is the tuberculin response (Tizard, 2000).

1.6.1 The tuberculin reaction

The tuberculin reaction is an immunological specific inflammatory reaction mediated by T-cell. Tuberculin is the name given to extracts of mycobacterium tuberculosis, *Mycobacterium bovis* or *Mycobacterium avian.*

The most important type of tuberculin is purified protein derivative (PPD) tuberculin, which is prepared by growing organisms in synthetic medium, killing them with steam and filtering. When tuberculin is injected into skin a delayed hypersensitivity response will occur. Following intra dermal injection of tuberculin into animal, a red, indurate (hard) swelling slowly develops at injection site. The inflammation begins between 12 and 24 hours and reaches its greatest intensity between 24 to 72 hours, and persists for several weeks before gradually fading.

Histological examination of the lesion shows that it is infiltrated with mononuclear cells (lymphocytes, macrophages); although a significant neutrophil accumulation may be present in the early hours of the reaction (Tizard, 2000).

CHAPTER TWO
MATERIALS and METHODS

2.1 Experimental Animals

Sixteen healthy male rabbits weighing (1000-1090 gm), one and one and half years of age, were purchased from the local market at Omdurman. These rabbits were housed in cages (120×100cm) in the research laboratory at the Biochemistry Department, Faculty of Veterinary Medicine - University of Khartoum.

The rabbits were divided into four experimental groups each containing four animals .Two groups were injected intravenously (I.V) with 150 mg kg $^{-1}$ of 10% alloxan (Sigma Chemical Co., St Louis, Mo ,USA) dissolved in Isotonic Na cl to induce diabetes mellitus at the start of the experiment . The other two groups were injected with the same volume of isotonic Na cL in a similar way to the diabetic groups .Three days after alloxan – injection, diabetes was confirmed by the demonstration of hyperglycemia (blood glucose >200mg/dl). Rabbits were not treated with insulin at any time during the experiment (Yegen *et al*.,1995).

All animals were fed with crushed –sorghum and Lucerne and given clean drinking water. Rabbits were adapted for 15 days before starting the experiment. And then were fed the experimental diet for four weeks.

2.2 Feeding program

The animals were allotted to four different groups, each of four rabbits.

- Group A: non - diabetic (control) group fed (0 % *Nigella sativa*) preparation composed of 100 gm crushed sorghum plus Lucerne.
- Group B: non - diabetic received 15 % (w/w) preparation composed of 85 gm .crushed sorghum and 15 gm black cumin plus Lucerne.
- Group C: diabetic (control) fed 0 % *Nigella sativa*) preparation composed of 100 mg crushed sorghum plus Lucerne.

-Group D: diabetic received 15 % (w / w) preparation composed of 85 mg crushed sorghum and 15 gm of black cumin plus Lucerne. *N. sativa* seeds were obtained from a commercial source in the local market. The viability of the seeds was confirmed by germination test according to (Ista, 1979).

Collection and preparation of blood samples

Fresh blood samples (3ml) were collected from the jugular vein of the rabbits using disposable syringes, into clean glass tubes using sodium citrate as anticoagulant .These samples were collected for the determination of blood glucose concentration.

For the determination total serum proteins, albumin and globulins, 3 ml of blood were taken using disposable syringes. Then serum was separated and stored frozen at -20 c° until analyzed for total serum proteins, albumin, and globulins.

Collection was done after using all the precautions of handling the animal *i.e*. shaving, sterilization with alcohol % and avoiding sampling from an excited animal.

Blood samples were collected before induction of diabetes mellitus to measure the normal values, then after the induction diabetes immediately, then collected twice every 15 days for one month.

Fresh blood samples for phagocytosis determination were collected after feeding *N. sativa* for four weeks.

2.2 Biochemical Analysis

2.2.1 Determination of Blood Glucose (oxidase method)

It is an enzymatic method that measures the glucose concentration in blood and serum according to (Trinder, 1969).

Principle

Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid .The formed hydrogen peroxide (H_2O_2) is detected by a chromogenic oxygen acceptor, phenol –aminophenazone in the presence of peroxide (POD).

B-D-Glucose $+O_2+H_2O \xrightarrow{GOD}$ Gluconic acid + H_2O_2

H_2O_2 + phenol + Aminophenazone $\xrightarrow{POD}$ Quinoneimine + H_2O

The intensity of the color is proportional to the glucose concentration in the sample.

Reagents

Reagent (1): working reagent (R_1): Buffer phenol 0.3 m mol /l, TRIS Ph 7.4

Reagent (2): (R_2) enzymes

Glucose oxidase (GOD) : 1500 U /L

Peroxidase (POD) : 1000 U / L

4-Aminophenazone (4AP) : 2.6 m mol / L

Dissolved contents of vial (R_2) enzymes in (R_1) bottle buffer.

Procedure

1. 1 ml of working reagent was pipetted into three test tubes, labeled test, blank and standard. 10 u l of sample was added to the test, and then 10 u l of standard was added to the standard tube.

2. The tubes were mixed and incubated for 10 minutes at 37 C°. The optical density (O-D) was read against a blank reagent at the wave length of 505 nm.

Calculations

Plasma glucose concentration was then calculated according to the following equation.

$$\text{Glucose (mg /dl)} = \frac{\text{(O-D) of sample} \times \text{standard concentration}}{\text{(O-D) of standard}}$$

Determination of serum total proteins

Serum total proteins were determined accordingly to the method of Reinhold, (1953).

Principle

Copper in alkaline solution react with peptide bonds that link amino acids in protein, producing a violet color. The intensity of the color is proportional to the concentration of protein present.

Reagents

1) Biuret reagent was prepared by weighing 9 gm sodium potassium tartarate then dissolved in 500ml of 0.2 N Na OH , to which 3 gm cupric sulfate were added.

2) Protein stock standard was prepared by dissolving 1 gm of crystalline (Bovine serum albumin) BSA and made up to 100 ml with distilled water (D.W.), a few drops of chloroform were added as preservative.

3) Working standard was prepared by diluting 10 ml of stock standard to 100 ml D. W to give concentration of 0.1 gm / 100 ml.

Procedure

Three test tubes labeled as test, blank and standard were prepared respectively. To all tubes 5 ml of biuret reagent was added. To the test tube 0.2 ml of serum, then 2.8 ml D.W were added .For the standard tube 3 ml of working reagent were added. All tubes were mixed and incubated for 10 minutes at 37 C° a water bath .The (O –D) was read at 540 nm against a reagent blank.

Calculations

The concentration of serum total protein was calculated as follows:

$$\text{Protein concentration (g/dl)} = \frac{(O - D) \text{ of sample} \quad \text{standard concentration}}{(O - D) \text{ of standard}}$$

Determination of serum albumin

Serum albumin was determined by the method of Bartholomew and Delaney (1967) using Bromo-cresol green (BCG).

Principle

Bromo-cresol green (BCG) solution, a yellowish indicator, reacts with serum protein producing a blue green color. The color intensity was proportional to the concentration of protein present.

Reagents

(1) Molar solution of sodium citrate, was prepared by dissolving 29.4 gm tri-sodium citrate in DW, and completed to100 ml.

(2) Molar solution was prepared by dissolving 21 gm citric acid in 100ml DW.

(3) Bromo-cresol green (BCG) 0. 1M was prepared by adding 0.174 gm of BCG, to 2.5 ml of (0.01N) Na OH then the volume completed to 25 ml with D.W.

(4) Albumin standard was prepared by dissolving 4 gm of BSA in 100 ml DW and kept at -20 c°.

(5) Working BCG solution was prepared by mixing 17.3 ml tri-sodium citrate , 32.7 ml citric acid and 6.o ml (0.1 M) BCG , the volume is made up to one liter , the pH was adjusted to 3.8 to 4 and kept in brown bottle at 4 C° .

Procedure

4 ml BCG solution were pipetted into three test tubes labeled; test, blank, and standard. 0.02 ml of serum was added to test tube followed by 0.02 ml then DW.

The test tubes were mixed and the (O.D) was read against areagent blank at the wave length of 360 nm.

Calculations

Serum albumin concentration was then calculated according to the following equation:

$$\text{Albumin concentration (g/dl)} = \frac{\text{(O. D) of sample} \times \text{standard concentration}}{\text{(O. D) of standard}}$$

Determination of total serum Globulins

Serum globulins were calculated according to Wunderly and Wuhtmann, (1960) by subtracting albumin from serum total proteins.

2.4 Phagocytosis Experiment

Phagocytosis of *Staphylococcus aureus* by rabbit polymorpho neutrophils, in the presence of opsonin was measured using the method described by (Mohammed, 1993).

Media Preparation

1- Nutrient broth (Oxoid Ml England) were added to one liter DW, mixed thoroughly and distributed in five ml volume by autoclaving at 121 C°. For 15 minutes.

2- Blood agar (Cowan and Steal, 1985), 40 gm blood agar base were dissolved in one liter DW, boiled to dissolve completely, mixed and sterilized by auto- calving at 121 C° for 15 minutes. Aftercooling at 45 C° defibrinated sheep blood was added at the rate of 7.5 % a septically. The medium was then poured in to plates in 15 ml aliquots and left to solidify under completely a septic condition.

Preparation of McFarland Nephelometer standard

Principle

A chemically induced precipitation reaction is used to approximate the turbidity of bacterial suspension.

Method

Standard tube no. (4) was prepared by adding (9.5) 1% $H_2 SO_4$ to (0.5 ml) 1 % barium chloride and mixed thoroughly, the turbidity of the suspension was equivalent to the concentration of bacteria (1.2×10^9).

Storage of McFarland standard tubes in the dark at room temperature should be stable for six months (Baron and Lance, 1994)

Phagocytosis Procedure

-1- The bacteria were grown in 5 ml nutrient broth at 37 Cº for 24 hours, under increased CO_2 tension and used at concentration equivalent to Mcfarland opacity tube 4; (1.5×10^9).

-2- A volume of 0.9 ml sterile normal saline was pipette into three test tubes under complete aseptic condition. 0.1 ml of cultured *Staphylococcus aureu*s was added to the first tube then serially diluted. The concentration used was 1/1000

-3-Three micro - centrifuge eppendorf tubes were used, in the first one 0.2 ml of the diluted organism was added to 0.1 ml of tested rabbit serum (non-diabetic treated, Group B), and in the second eppendorf 0.2 ml of the diluted organism and 0.1 ml of control rabbit serum (non-diabetic untreated, Group A). The tubes were incubated for half an hour with mixing every 10 minutes, then 0.5 ml fresh rabbit blood was added to each tube. The third ependorf tube was used as negative control containing 0.2 diluted organisms with 0.6 normal untreated rabbit sera.

Phagocytosis assay was carried out by the incubation of the micro centrifuged ependorf tubes with frequent mixing for one hour at 37 C°.

The mixture of each test was plated by dropping 10UL into fresh sheep blood agar plates.

Each experiment was done in triplicate, the plates were then left to dry under complete aseptic condition and incubated at 37 C° for 24 hours, and under increased CO_2 tension growing colonies were then counted and compared .

The same methods repeated with diabetic treated and diabetic untreated rabbits.

Hypersensitivity Experiment

2.5 Tuberculin Skin Test- Method (Mantoux Skin test)

This test is to detect the immunological response resulting from infection with *Mycobacterium tuberculosis*.

After the rabbits had been fed the experimental diet for four weeks, all groups were injected with 0.1ml tuberculin, this clear solution colorless to pale yellow, contains

cleaned proteins (purified protein derivative) from *Mycobacterium tuberculosis* .It is injected intradermaly at the flank site, after shaving the area and thoroughly disinfected with cotton swap soaked in alcohol, a circle is drown at the middle of the shaved area, and skin fold was then taken and injected intradermaly, using small syringes (1ml).

The skin thickness was measured by means of a caliper; before injection and twice after 24and 48 hours after injection and the results are recorded and compared.

Statistical Analysis

Factorial completely randomized design was used for analysis of glucose , proteins ,albumin , globulins and tuberculin test (Gomez and Gomez, 1984).

Means separation was done according to Duncan's Multiple Range test (DMRT) (Gomez, and Gomez, 1984).

Phagocytosis test was done by student T. test (Mendehall, 1971).

CHAPTER THREE

RESULTS

3.1 Blood Parameters

3.1.1 Blood glucose

The effect of feeding *N. sativa* on plasma blood glucose concentration is represented in Table (1) Fig (1).

Two diabetic groups C and D showed significantly higher level of total mean of blood glucose concentration ($p<0.05$) compared to the other normal groups A and B, after feeding *N. sativa* for two weeks, to groups B and D, no significant difference observed in the two groups compared to time zero. Whereas diabetic groups still have significantly higher glucose level than non- diabetic groups ($p< 0.05$). The same manner was also observed at week four. Normal treated group showed slight decrease in blood glucose concentration but not significant compared to week 2, where as blood glucose concentration increase significantly ($p<0.05$) in group D.

Group C, (diabetic untreated) reached high level of 371.90 ± 55.70 g /dl blood glucose concentration at week 4.

The effect of feeding *N. sativa* on the level of plasma blood glucose clearly observed in group B, which showed slight non significant decrease in glucose concentration at week four compared to zero time.

Table (1) the effect of feeding 15 % (w/w) of *N. sativa* on blood glucose concentration (mg/ dl) of local rabbits (Means ± standard error).

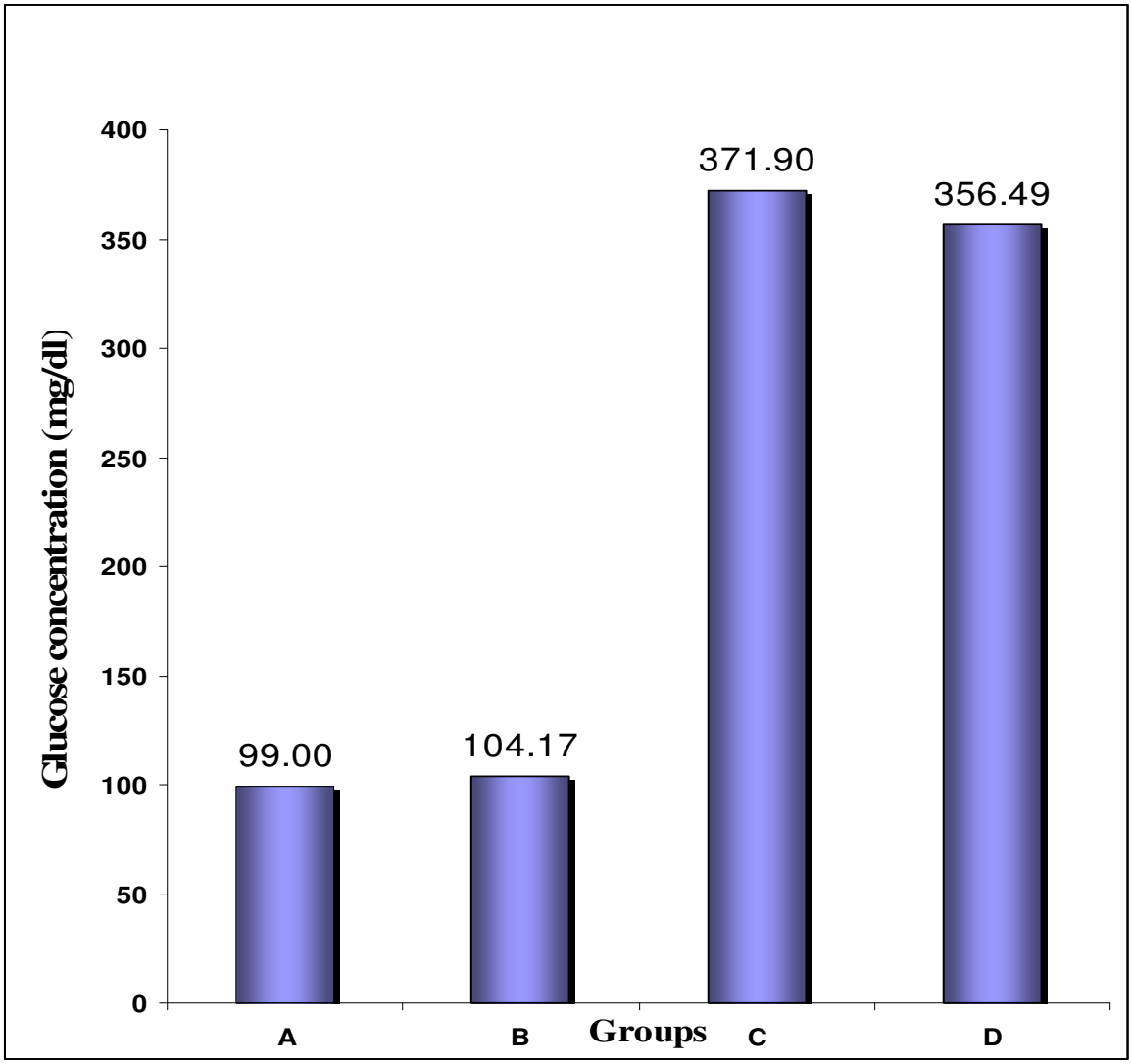
N=4

Groups	Zero time	Weeks 2	Weeks 4
A	133.37 A a ±21.99	117.30 A a ±26.17	99.00 A a ±1.79
B	121.95 A a ±5.55	110.21 A a ±9.25	104.17 A a ±19.85
C	260.17 B a ±31.67	329.65 B a ±41.58	371.90 B a ±55.70
D	269.67 B a ±31.67	322.33 B a ±13.62	356.49 B a ±21.35

A = Non diabetic untreated group B = Non diabetic treated group
C = Diabetic untreated group D = Diabetic treated group

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (1) The effect of feeding 15% of *N. sativa* (w/w) on blood glucose concentration (mg/ dl) after four weeks



3.1.2 Total Proteins

The effect of feeding 15% of *N. sativa* on total serum proteins concentration is represented in Table (2) Fig (2).

The untreated groups A and C, showed low total mean values of protein 6.70 ± 0.17 and 6.92 ± 0.34 g/dl for group A, which was non-diabetic untreated, and group C, which was diabetic untreated respectively. Treated group (B) showed high levels of total protein's 7.53 ± 0.51 g/dl. The diabetic treated group (D), showed total mean value of 7.21 ± 1.09 g/dl which was slightly higher compared to the untreated groups (A and C). After the first two weeks the treated groups B and D showed elevation in serum total protein concentrations. The elevation continued to week 4, when group B recorded significant increase ($P < 0.05$).Group A and C maintained the same level until the end of the experiment.

Table (2) the effect of feeding 15% (w/w)) of *N. sativa* on total serum proteins (g/dl) of local rabbits (Means ± standard error).

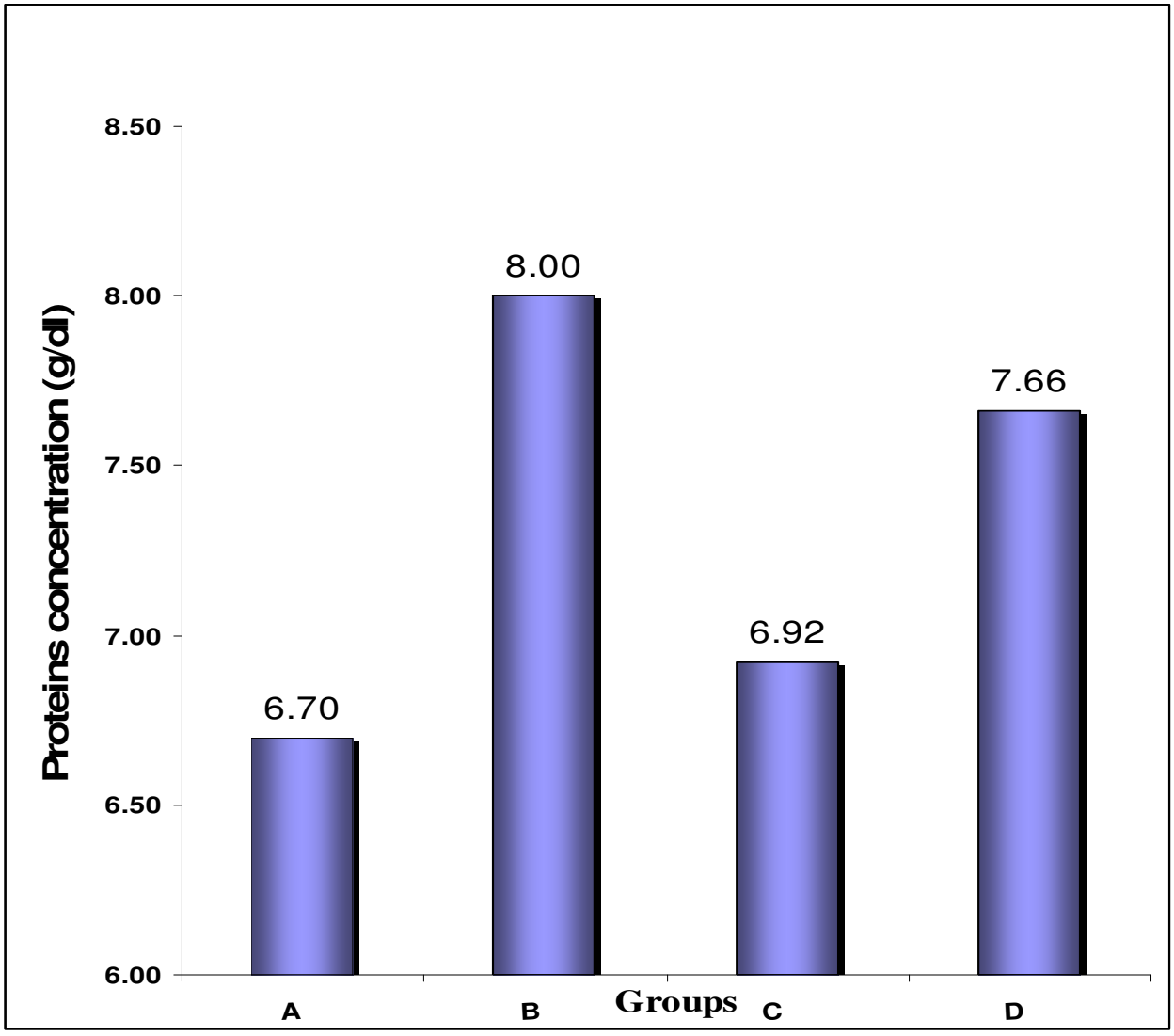
N=4

Groups	Zero time	Weeks 2	Weeks 4
A	6.45 A a ± 0.61	6.88A a ± 0.34	6.70 Ba ± 0.17
B	7.06 Ab ± 0.71	7.54 Aa ± 0.28	8.00Aa ± 0.31
C	7.27 Aa ± 0.17	6.29 Aa ± 0.15	6.92 Aa ± 0.34
D	6.55 Aa ± 0.16	7.41 Aa ± 015	7.66 Aa ± 0.62

A = Non diabetic untreated group B = Non diabetic treated group
C = Diabetic untreated group D = Diabetic treated group

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (2)The effect of feeding 15% of *N. sativa* (w/w) on total serum proteins (g/dl) after four weeks



3.1.3 Total Serum Albumin

The effect of feeding 15% of *N. sativa* on serum albumin concentration is represented in Table (3) Fig (3).

During the experiment, groups A and C showed fluctuating values within the four weeks without clear increase, in contrast to the treated groups.

The untreated groups A and C showed low albumin concentration of 3.79 ± 0.07 and 3.83 ± 0.02 g /dl respectively. The treated groups showed high serum albumin levels; group B recorded 5.02 ± 0.19 g /dl, group D showed 5.00 ± 0.04 g /dl. So treated groups showed significantly elevated total serum albumin concentration started at week 2, ($P < 0.05$), and continued to the last week, which were significantly higher than the zero time and week two. Treated non diabetic groups, showed high level than treated diabetic ones.

Table (3) the effect of feeding 15 % (w/ w) of *N. sativa* on serum albumin (g /dl) of local rabbits (Means ± standard error).

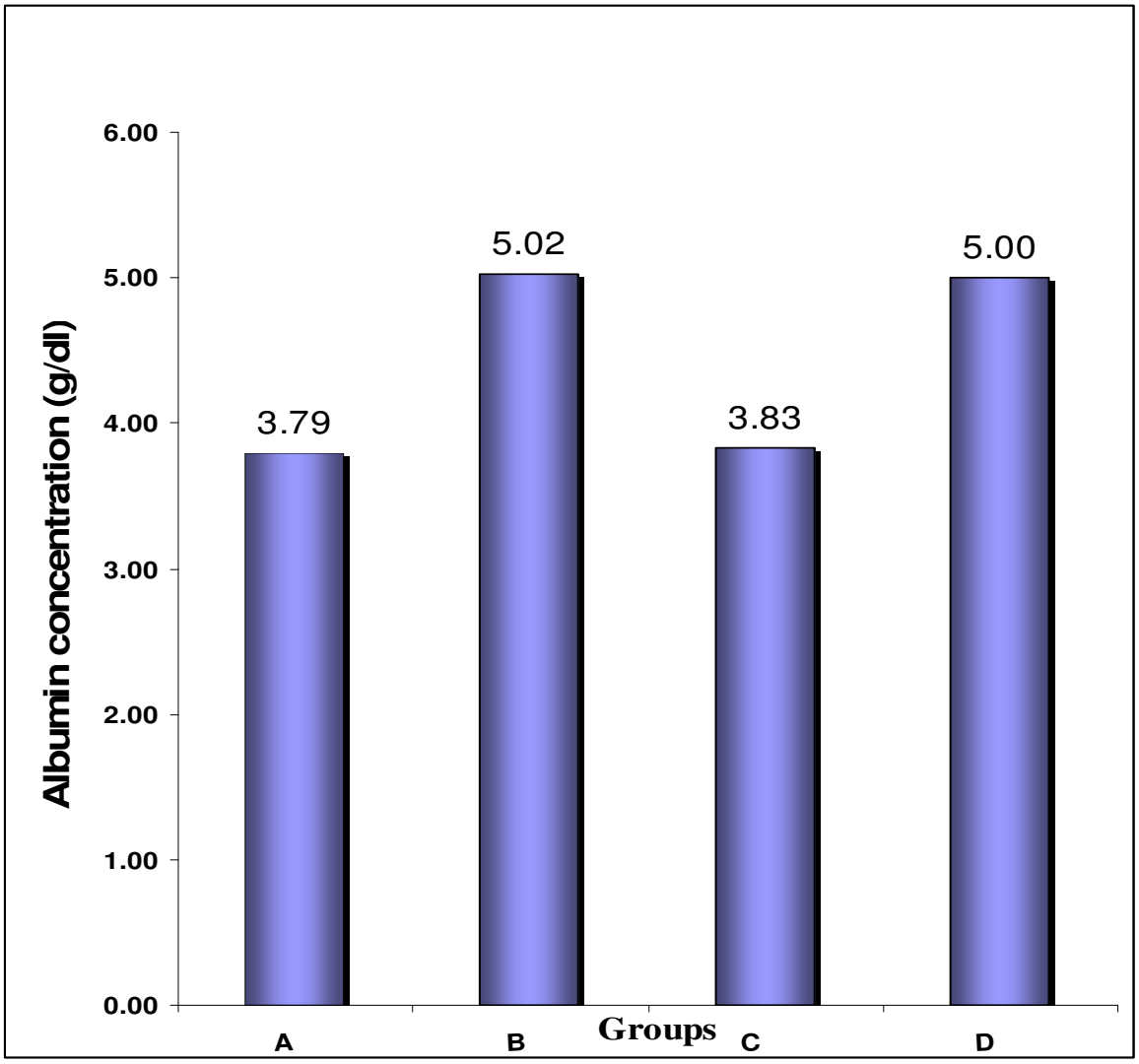
N=4

Groups	Zero time	Weeks 2	Weeks 4
A	3.44 Aa ± 0.04	3.93 Ba ± 0.04	3.79 Ba ± 0.07
B	4.30 Ab ± 0.59	5.16 Aa ± 0.07	5.02 Aa ± 0.19
C	3.62 A a ± 0.29	3.40 Ba ± 0.02	3.83 B a ± 0.02
D	4.08 Aa ± 0.08	4.91 Aa ± 0.04	5.0 A a ± 0.04

A = Non diabetic untreated group B = Non diabetic treated group
C = Diabetic untreated group D = Diabetic treated group

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (3) The effect of feeding 15% of *N. sativa* (w/w) on total serum albumin (g/dl) after four weeks



3.1.4 Total Serum Globulins

The effect of feeding *N. sativa* on serum globulins concentration is represented in Table (4) Fig (4).

The control groups A and C showed mean values of 2.73 ± 0.16 and 3.09 ± 1.05 g/dl respectively.

Groups B and D showed mean value of 2.98 ± 0.77 and 2.56 ± 0.27 g/dl respectively.

In all groups, treated and untreated no significant effect on serum globulins was observed.

Table (4) the effect of feeding 15 % (w/w) of *N. sativa* on total serum globulins (Means ± standard error).

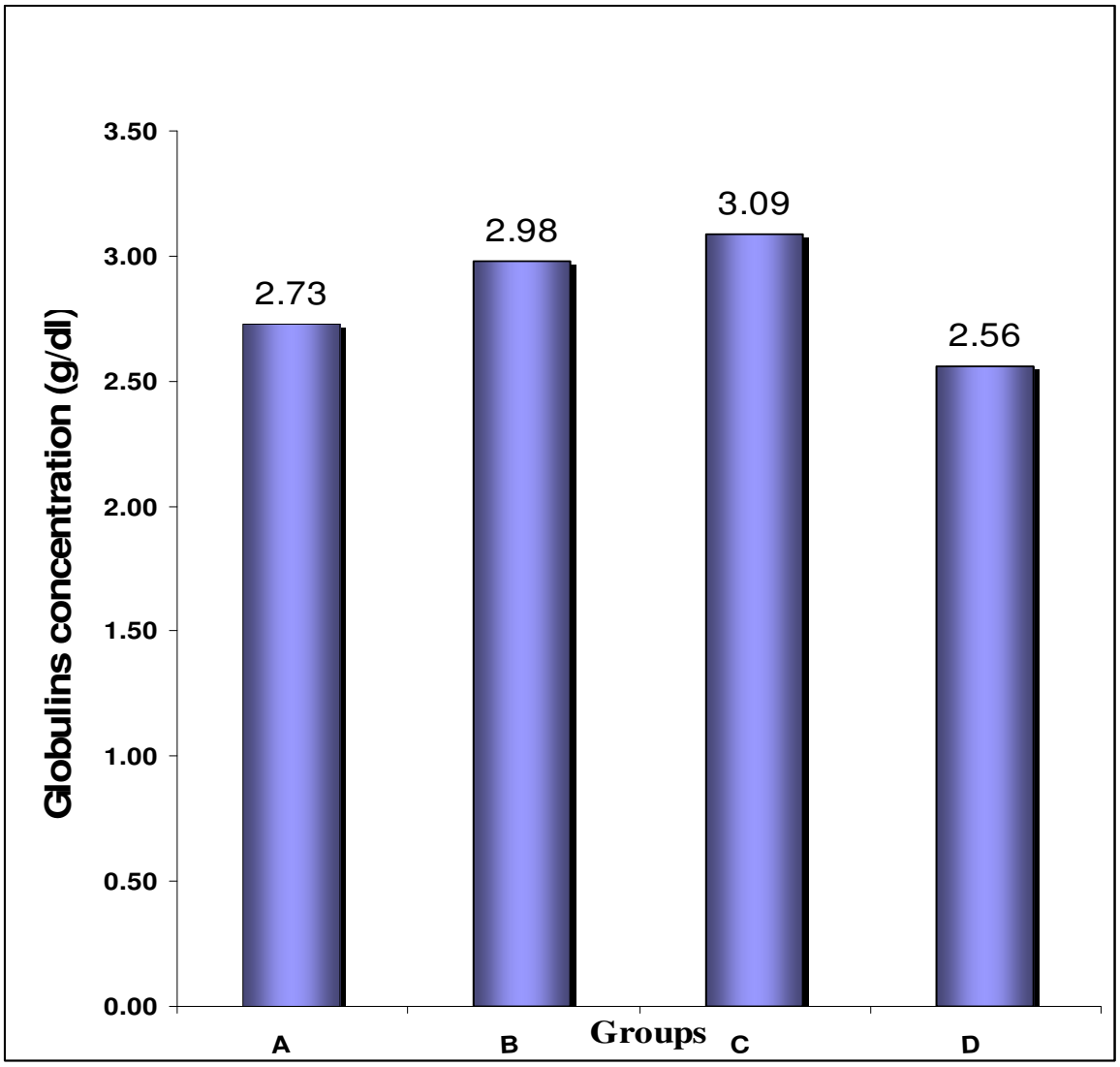
N=4

Groups	**Zero time**	**Weeks 2**	**Weeks 4**
A	3.01 Aa ± 0.04	2.93 Aa ±0.05	2.73 Aa ± 0.16
B	2.76 Aa ± 0.29	2.38 Aa ±0.33	2.98 Aa ± 0.77
C	2.65 Aa ± 0.35	2.89 Aa ± 0.52	3.09 Aa ± 1.05
D	2.48 Aa ± 0.61	2.51 Aa ± 0.42	2.56 Aa ±0.27

A = Non diabetic untreated group B = Non diabetic treated group
C = Diabetic untreated group D = Diabetic treated group

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (4) The effect of feeding 15% of *N. sativa* (w/w) on total serum globulins (g/dl) after four weeks



3.2 Immune reactions

3.2.1 Phagocytosis test

Table (5) and Figure (5) present the results of phagocytic activity for all groups of animals as colony forming units (CFU).

Phagocytic activity, in the four groups, showed decrease in the number of the colonies significantly when compared to the control ($P< 0.05$).

The non diabetic groups presented significant lower number of colonies compared to diabetic ones of all treated and untreated ($P< 0.05$).

Also treated groups, showed lower number of colonies compared to non treated groups.

The highest phagocytic activity was observed in the treated non diabetic group (99.33 ± 16.37 colonies), and the lowest effect was observed in the diabetic non treated group (225.67 ± 2.96 colonies). The difference was significant ($P< 0.05$).

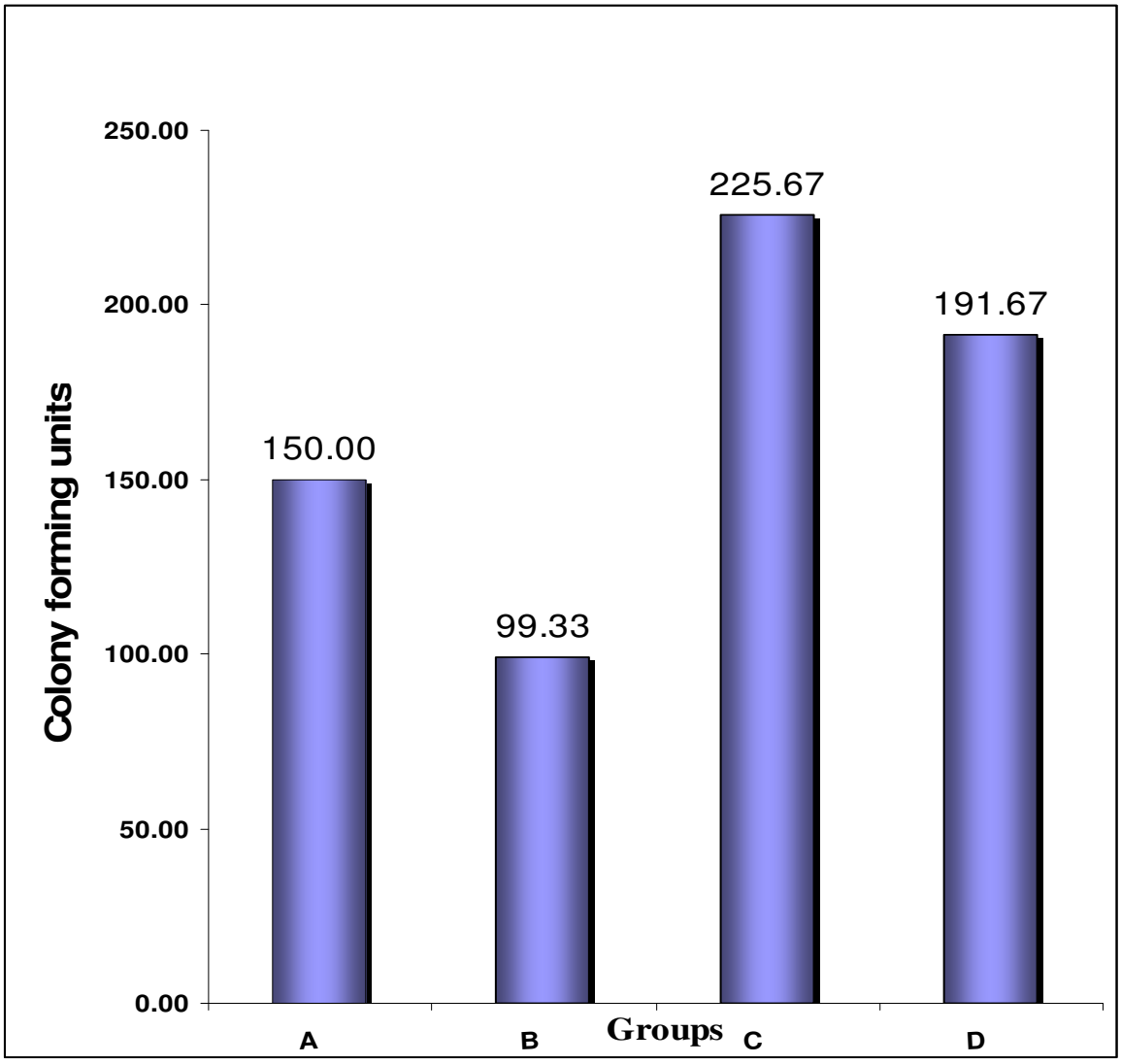
Table (5) the effect of feeding 15 % (w/w) of *N. sativa* on phagocytic activity (colony forming units). (Means ± standard error).

N=4

Groups	**Untreated** Staph.+ serum (control test)	**Untreated** Staph.+ serum+ blood	**Treated** Staph.+ serum+ blood
Non-diabetic	245.67 ± 2.33Ba	**Group A** 150.00 ±2.88Bb	**Group B** 99.33 ± 16.37 Bc
Diabetic	272.00 ± 6.11Aa	**Group C** 225.67±2.96 Ab	**Group D** 191.67 ±6.01 Ac

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (5) The effect of feeding 15% of *N. sativa* (w/w) on Colony forming units after four weeks



3.2.2 Tuberculin Reaction

The effect of feeding *N. sativa* on tuberculin reaction is represented in Table (4) Fig (4).

In tuberculin reaction test, the thickness of skin is measured three times, one before injection, second after 24 hours of injection and the last one after 48 hours.

All groups showed increase in skin thickness at 24 hours, and 48 hours after the injection.

Non diabetic groups showed low skin thickness compared to diabetic groups.

Untreated groups A and C showed 0.29 ±0.03 and 0.31 ± 0.06 cm. for A and C respectively, after 48 hours.

Treated groups B and D showed low increase thickness after 24 hours (0.25 ± 0.03 and 0.28 ± 0.03 respectively) and 48 hours compared to non treated controls.

The least skin thickness increase was observed in treated non diabetic group B.

Table (5) The effect of feeding 15% of *N. sativa* (w/w) on tuberculin reaction (thickness of skin) of local rabbits (Means ± standard error).

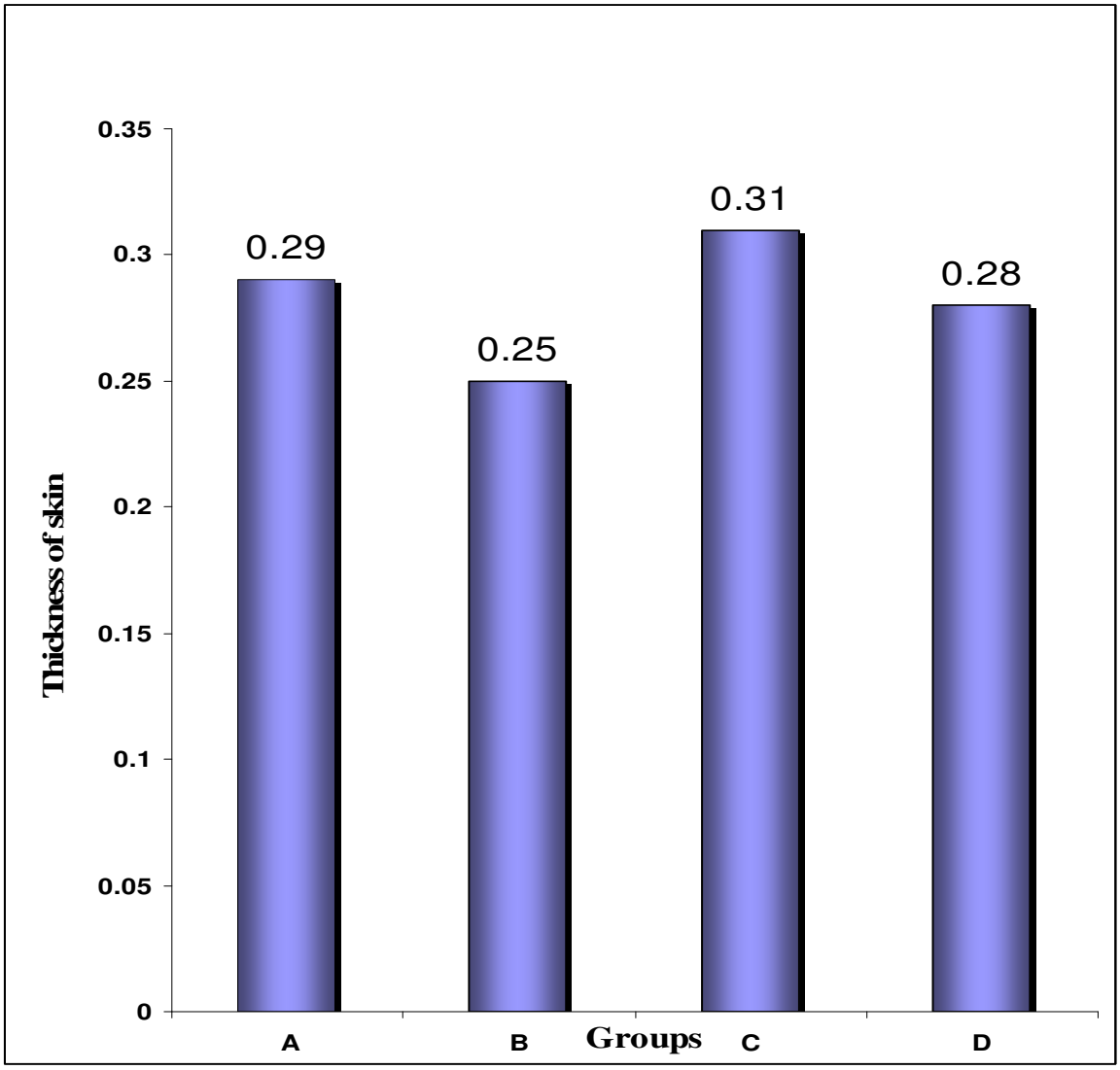
N=4

Groups	Before injection	After 24 hrs of injection	After 48 hrs of injection
A	0.24 Aa ± 0.04	0.27 A a ± 0.05	0.29 A a ±0.03
B	0.22 A a ±0.03	0. 23 A a ± 0.01	0.25 A a ± 0.03
C	0.24 A a ± 0.04	0.27 A a ± 0.03	0.31 A a ± 0.06
D	0.23 A a ±0.05	0.25 A a ±0.03	0.28 A a ± 0.03

A = Non diabetic untreated group B = Non diabetic treated group
C = Diabetic untreated group D = Diabetic treated group

Means in the same columns followed by different capital letters are significantly different at $P < 0.05$.
Means in the same rows followed by different small letters are significantly different at $P < 0.05$.
n = number of replicates

Figure (7) The effect of feeding 15% of *N. sativa* (w/w) on Hypersensitivity test (thickness of skin) after 48 hours of injection



CHAPTER FOUR
DISCUSSION

The current study was performed in non-diabetic and induced diabetic rabbits to investigate the effect of feeding whole *N. sativa* seeds on plasma glucose, proteins and some immune reactions (phagocytic activity and hypersensitivity reaction).

Data on using the whole *N. sativa* seeds is lacking. Previous studies concentrated on extraction of *N. sativa* oil, and *N. sativa* mixed with other antidiabetogenic plants.

4.1Blood glucose

The effect of feeding *N. sativa* seeds to non diabetic and diabetic rabbits was studied, diabetes was induced by administration of alloxan to the rabbits , until blood glucose was elevated to (>200mg /dl).The result showed slight decrease in blood glucose in the non diabetic treated group and no effect on glucose concentration was observed in diabetic ones , Table (1).

Al Wadi *et a*l., (1991), used mixture of *N. sativa, Myrrh, Gum olibarum, Gum asafetida* and Aloe. The mixture showed hypoglycemic effect in non diabetic and diabetic rabbits. The antidiabetic action of the plant extract may be mediated through decreased hepatic gluconeogenesis .Also ELDakhakhny *et a*l., (2002) investigated the effect of plants mixture containing extract of *N. sativa* and he reported lowering effects of the mixture on diabetic rabbits. In other study using a mixture of five plants, only the extract of myrrh and aloe gums effectively increased glucose tolerance in both normal and diabetic rats, while other plants like *Gum olibarum* and *N .sativa* had no effect on glucose concentration, (Al wadi and Gumaa, 1987).

Results reported in the present work were in agreement with those found by (ELNaggar and ELDeib, 1992) who mentioned that oral administration of powdered *N. sativa* seeds for three weeks produced minimal insignificant reduction in blood glucose in normal and alloxan –induced diabetic rabbits.

Many authors reported that *N. sativa* seeds had hypoglycemic effect *(Le et a*l., 2004). The petroleum ether extract of *N .sativa* seeds was reported to have hypoglycemic effect (Richid *et a*l .,2004) .Oral administration of *N. sativa* extract decreased blood glucose significantly (Meral *et a*l .,2001).Observations of the hypoglycemia effect of *N .sativa* oil in diabetic rabbits were recorded by (Fararh *et a*l .,2004) .

All these findings support the use of oil extract of *N. sativa* because it is more effective in decreasing blood glucose more than whole seeds. It was proved that it gave good results when mixed with other antidiabetogenic plants. Many studies were needed to compare different preparation of *N .sativa* by different ways of administration in order to explain their effects.

4.2 Serum total Proteins

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin deficiency and /or insulin resistance.

The role of insulin is to stimulate the uptake of amino acids into muscle and increases proteins synthesis. It also decreases protein catabolism and inhibits the oxidation of amino acids in the liver. Whereas insulin deficiency causes wasting due to increased break down and reduced syntheses of proteins (Rang, 1999).

In the present study the effect of feeding 15% of *N. sativa* seeds on the serum total proteins was obvious in the two treated groups compared to the control groups that ,the seeds caused significant ($P< 0.05$) increase in serum total proteins whereas group B showed high levels than group D which was diabetic and treated .The elevation started after two weeks and continued to week 4 where high level of total proteins was recorded compared to zero time .These findings agreed with the results of (Al-Gaby,1998) who found that feeding Nigella cake proteins significantly increase the serum total protein and its two fractions albumin and globulin. Furthermore, study by (Omer, 2001) found increase in total serum proteins and its two fractions albumin and globulin, when fed 15% of *N. sativa* to rabbits.

During the experiment groups A and C showed fluctuating values within the four weeks without clear increase, in contrast to the treated groups. However *N. sativa* increased the total serum proteins.

Badary *et al*., (2000), used the thymoquinone (TQ), the main constituent of the volatile oil of *N. sativa* seeds as a protective agent for proteinuria and albuminuria.

4-3 Serum total albumin

In the present study the effect of feeding 15% of *N. sativa* seeds on the serum total albumin was obvious in the two treated groups compared to the control groups. The seeds caused significant increase in serum total albumin whereas group B showed high levels than group D which was diabetic and treated .The elevation started after two weeks and continued to week 4 where level of total proteins was high compared to zero time .This finding agrees with the results of (Al-Jishi and Abuo-Hozaifa, 2003) who found that feeding *Nigella sativa* significantly increase the total albumin. Furthermore, (El-Hag, 1998) reported that the consumption of the seed may improve the production of immunoglobulins and synthesis of albumin in the liver.

4-4 Serum total globulins

In the present study the effect of feeding 15% of *N. sativa* seeds on the serum total globulins was observed in the two treated groups compared to the control groups; that the seeds caused slight increase in serum total globulins (group B showed higher levels than group D).The elevation started after two weeks and continued to week 4 where high levels of total globulins were recorded compared to zero time .This finding agrees with the results reported by Omer, 2001.

4-4 Phagocytosis test

In the present study the phagocytosis by rabbit's neutrophils was tested *(in vitro*) in four different groups of animals, divided into diabetic and non-diabetic groups, one of each had been fed *N. sativa*. It was noticed that all groups fed *N. sativa* developed

higher phagocytic activity, and non diabetic groups recorded insignificant higher level than diabetic groups. This agrees with (Osar, *et a*l .,2004) who found that the phagocytic activity was lower in diabetic patient when compared to healthy subjects , but the difference was not statistically significant, but it was correlated with lower oxidative indexes when compared to healthy controls. Furthermore, the neutrophil function is impaired in patients with diabetes mellitus. Other studies in uncontrolled hyperglycemia demonstrated that the uncontrolled hyperglycemia caused impairment of host defense including decreased in polymorphonuclear leukocyte mobilization, chemotaxis, and phagocytic activity (Butler *et al*., 2005).

When the two diabetic groups were compared, it was found that group D, which was treated with *N. sativa* had lower colony forming units which indicate higher phagocytic activity. This agrees with the findings by (Kanter *et a*l., 2004), who reported that treatment with *N. sativa* exerted therapeutic protective in diabetes by decreasing oxidative stress so preserving pancreatic beta-cell integrity. In addition to that antioxidant enzymes activity was increased.

The present findings suggest that elevated phagocytic activity in non-diabetic, more than, diabetic ones could be due to dysfunction of neutrophils, because diabetes mellitus has an increase effect on oxidative enzymes activity and thus phagocytic cell activity. Feeding *N. sativa* may improve activity of neutrophils by increasing antioxidant enzymes activity, and stimulation of phagocytic cells either directly by number's or via factors that affected the phagocytic activity as opsonins, which consist of antibodies and complements .

4-5 Hypersensitivity reaction

Mycobacterium tuberculosis has ability to initiate delayed cutaneous hypersensitivity, via its components of the cell wall such as proteins and polysaccharides which both have immunogenic potential. It was proved that the polysaccharides act as the key epitopes for cellular immunity. (Terr and Stites, 1991).Immunization by proteins

derived from cultures *M. tuberculosis* stimulated cell mediated immune response and these lead to infiltration of mononuclear cells and release of various cytokines (Rang,1999).

In the present study all the immunized groups were characterized by a reddening of skin and a localized injurious reaction which reach its height after 24-48 hours.

All treated groups showed decreases in skin thickness with disappearing of the redness and swelling after forty-eight hours. This agrees with (Omer, 2001)

The diabetic groups showed adverse skin lesion more than non-diabetic, but treated ones appeared with less inflammation in injected area compared to the untreated. The phagocytosis test showed higher phagocytic activity in all treated groups, and to some extend improvement of immunity in treated diabetic groups.

Appeared that *N. sativa*, acts as immunopotientiatng by increasing phagocytic activity as well as immunosuppressive factor for hypersensitivity reaction.

CONCLUSION

Feeding *N. sativa* to diabetic rabbits showed clear tendency to increase plasma proteins concentrations specially the albumin fraction. The phagocytic activity of the nutrophils was improved and the adverse effects of hypersensitivity reactions were reduced.

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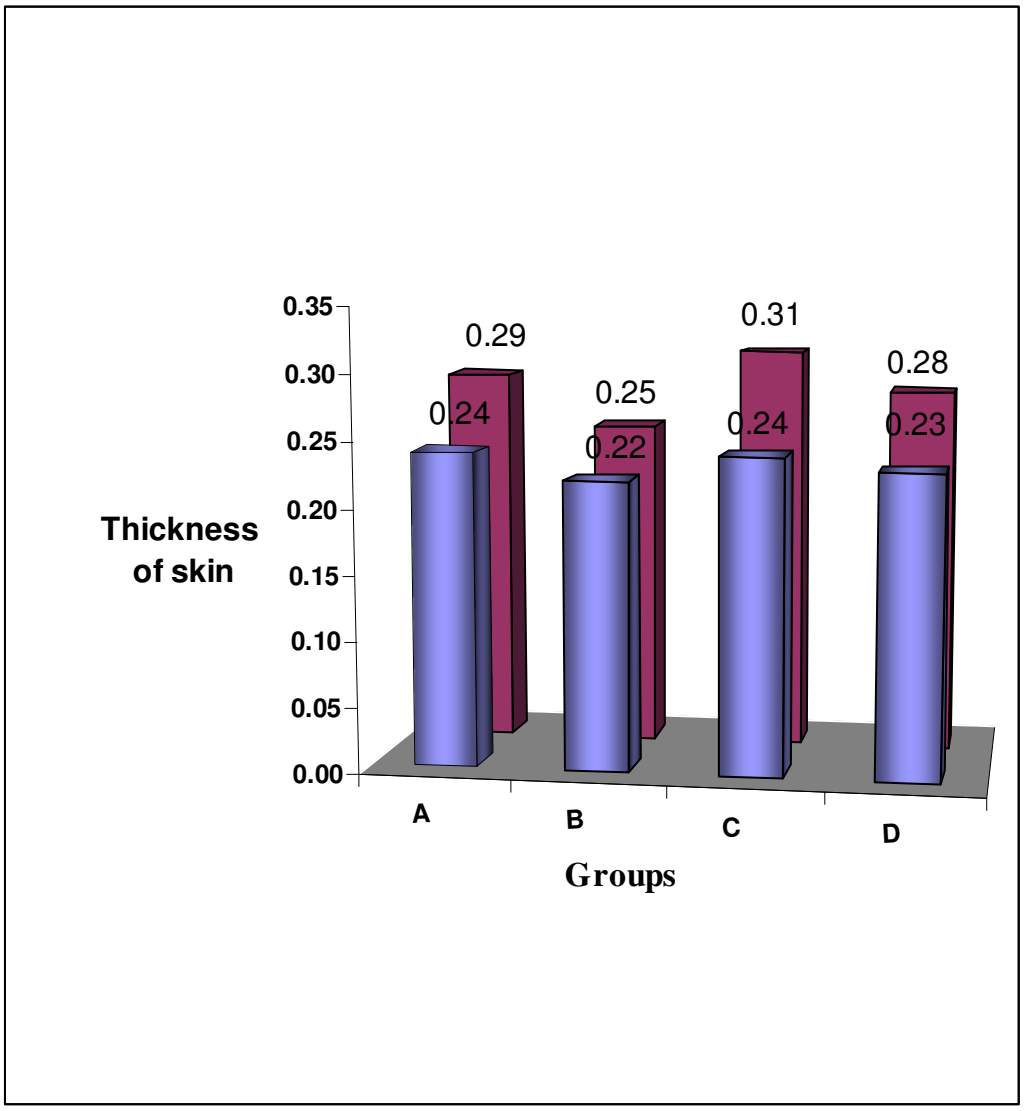
Figure (7) The effect of feeding 15% of *N. sativa* (w/w) on Hypersensitivity test (thickness of skin) Before and after 48 hours of injection


Thickness of skin
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.29
0.24
0.25
0.22
0.31
0.24
0.28
0.23
A
B
C
D
Groups